Annual Progress Report
Prepared by V. J. Wulff
NR: 119-266
Contract: Nonr 669(05)
Annual Rate: $6,290
Principal Investigator: V. J. Wulff
Research Associate: Manfred Brust
Title of Project: Electrophysiology of the Visual Pathway
Objectives: Study of coupling mechanisms in the retina

INSTITUTE OF INDUSTRIAL RESEARCH

DEPARTMENT OF ZOOLOGY

Syracuse University

Syracuse 10, New York

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I. Abstract

a) Since start of project:

From September 15, 1952, when this project started, until December 31, 1952, research equipment was made, incorporated into the present set-up and was calibrated.

b) During current report period:

The response magnitude and the latency of the retinal action potential elicited by a single flash of light has been determined for a wide range of light intensities and flash durations for grasshopper eyes. The response magnitude considered as a function of the logarithm of the flash duration, for a single intensity, exhibits for flashes of short duration a slowly rising phase, a linear rising phase at intermediate durations and a pleateau region for the longest flashes. The latency of the response is, for a single flash duration, a decreasing linear function of the logarithm of the light intensity as the intensity increases over most of the intensity range used in the experiments. As the flash duration becomes less than the latent period, for a constant intensity, the latency increases very slowly.

The experimental results can be described on the basis of a two factor theory which has been developed to correlate them. One factor which is generated when the light sensitive substance is exposed to illumination is responsible, after the lapse of the latent period, for the magnitude of the electrical response. The second factor or state whose generation is initiated by the light, but which is produced autocatalytically thereafter determines the latent period. The characteristics of the retinal action potential obtained from grasshopper eyes and from the median ocellus of Limulus are quantitatively described by the theory over practically the entire range of the measurements.

The response magnitude and latency of the retinal action potential elicited by a single flash of light has been determined for a wide range of light intensities and flash durations for the lateral eye of Limulus polyphemus, the horseshoe crab, and the frog, Rana pipiens. Except for deviations from theoretical predictions at higher intensities of stimulation, the results agree with those from the grasshopper.

The effect of temperature on the magnitude and latency of the retinal action potential of the grasshopper has been determined. The disparity in the temperature effect on the response magnitude and latency suggests that one process cannot control both the latency and magnitude. The effect of temperature on the magnitude of the retinal action potential supports the theoretical contention that the rising phase of the response vs. log flash duration plot is controlled by a photochemical reaction and that the plateau magnitude is controlled by a photochemical and a thermolabile reaction.

II. Plans for the future

a) Immediate

The effect of temperature on the reactions in photoreceptors leading to the electrical response is sufficiently interesting (see III-c) to merit exploration in eyes other than the grasshopper. The temperature studies are an indirect attempt to learn more about the coupling reactions in the visual process. We are ready to start work on the effects of various metabolites and drugs on the visual process.

An investigation of changes in spectral absorption of photosensitive cells following short but quite bright flashes of light will be attempeted. Such changes probably occur but whether they are large enough to be measurable is not yet known. In this connection we also wish to investigate the time characteristics of the bleaching process in rhodopsin.

b) Long Range

There seems little doubt that two types of reactions occur (see sections III-a, b and c) following illumination of photoreceptors, one controlling the potential magnitude and one controlling the latent period. Our long range program will be directed along lines to identify the reactions and to understand the reaction mechanism of the retina.

III-a

RETINAL ACTION POTENTIAL - THEORY AND EXPERIMENTAL RESULTS FOR GRASSHOPPER EYES*

V. J. Wulff**, W. J. Fry*** and F. A. Linde **

**Zoology Department, Syracuse University

***Bioacoustics Laboratory, University of Illinois

INTRODUCTION

When photoreceptors are illuminated a sequence of measurable changes occur which are instrumental in mediating the sense of vision. These changes are: 1) The absorption of radiant energy by a photolabile pigment or pigments and changes in the state(s) of the pigment. (Granit, '47; Wald, '51); 2) A potential change measurable across the retina with extra-cellular electrodes and called the retinal action potential (Hartline, Wagner and MacNichol, '52); 3) The initiation of nerve impulses in the axons of the sense cells (Hartline and Graham, '32; Granit, '47).

It has been frequently suggested (Hartline, '35; Wulff, '43; Granit, '47) that the retinal action potential is a generator potential producing local currents and initiating the trains of impulses in optic nerve axons. Recently it has been demonstrated (MacNichol, Wagner and Hartline, '53) with an intracellular electrode, that illumination causes a prolonged depolarization of the sense cells (presumably the eccentric sense cells) in the ommatidia of the Limulus lateral eye and that this depolarization causes the appearance of nerve impulse trains. The depolarization measured with the intracellular electrode presumably is the intracellular sign of the retinal action potential recorded with extra-cellular electrodes. Although the retinal action potential has been proved a generator potential only in the case of the lateral eye of Limulus, we subscribe to the general idea that retinal action potentials are generator potentials.

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* These studies were aided by a contract between the Office of Naval Research and Syracuse University, NR 119-266.

Between the photochemical event in vision and the production of the retinal action potential there occur processes and/or reactions which produce the potential change. It is the purpose of this report to describe some processes intermediate between the photochemical absorption of radiant energy and the retinal action potential and to compare these descriptions with data.

Method

The experiments were performed on dark adapted grasshoppers. The animals were securely fastened and a chamber was built about each of the two lateral complex eyes. These chambers were filled with 0.9% NaCl solution, and each chamber made contact with a calomel half cell through a salt bridge. The half cells were connected to the imput grids of a D.C. amplifier. The animal was so placed in a plastic container that the cornea of one eye could be exposed to a light flash admitted by the opening of a series of shutters, while the other eye remained in total darkness.

The interior of the chamber was ventilated by a constant stream of air at the temperature of water which circulated constantly through two copper heat exchangers built into the top and bottom of the animal chamber. Temperature was measured in terms of voltage developed by a copper-constantin thermocouple, with reference to a similar junction at 0° C, using a potentiometer circuit. Temperatures were regulated within $\pm$ 0.2° C of any given value. The air inside the chamber was approximately saturated with water vapor.

The animal chamber was securely fastened to a movable stage inside a Faraday cage, and the eye of the animal was oriented so that the cornea was at the focus of the light beam. The cage was then shielded from light and the animal was permitted to dark adapt.

Experiments were begun after 2 to 12 hours of dark adaptation. An interval of one hour between flashes proved to be necessary to permit complete recovery from the effects of preceding flashes. It was desirable to obtain a complete set of data from each animal; consequently the experiments were of several days

duration. The grasshoppers, however, were not adversely affected by the experimental treatment and even after two weeks in the experimental chamber, resumed a normal existence upon return to the colony.

After the temperature had reached the desired level the experimental procedure consisted of adjusting the intensity of the light to the desired level, using Wratten neutral filters, and admitting a flash of light of 100 microseconds duration.* The electrical response of the photoreceptor and of a photocell, to which a portion of the light beam had been diverted, were recorded by pens on moving paper and by photographing the display on the face of a 2-channel cathode ray tube. The paper receiving the ink tracing moved at 25.0 $\pm$ 0.25 cm. per second, and the sensitized paper photographing the cathode ray tube display moved at 50.0 $\pm$ 0.25 cm. per sec. After each record the deflection produced by a pulse of known voltage was recorded. A perforated disk driven by a synchronous motor past a light source supplied the time base of the camera. After an adequate interval for dark adaptation, another flash was admitted. Flash duration was varied by steps of 0.5 log units from 100 micro-seconds to 0.5 seconds. Intensity was varied by a factor of 10 from 10^{0} (unit intensity = 11,800 foot candles at the cornea) to 1×10^{-4}.

Experiments were also performed on the isolated median ocellus of Limulus. The technique used has been described (Wulff and Pandazi, '51). The isolated ocellus preparation gave reproducible responses for about 8 hours at 15° C. The relatively short life of the preparation necessitated shortening the experimental procedure. Consequently complete sets of data were not obtained from any one preparation. Except for curtailing the procedure, the experiments were conducted as on the grasshoppers.

A total of 16 experiments were performed on grasshoppers and 12 experiments on isolated ocelli. Although all the data obtained exhibited similar relations,

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* The high speed shutter was designed by Frank J. Fry and assembled under his supervision. We express our appreciation to him.

single sets of data were selected for the text.

The measurements of magnitude and the latency were made as follows: 1) The base line was extended below the response and the points of greatest deflection found and measured and the voltage computed; 2) The point where the tracing could be seen to leave the base line was marked as the end of the latent period and the interval from onset of illumination to this point was measured and converted to seconds. The error has been estimated at $\pm$ 10% at low intensities or short exposures and $\pm$ 5% at high intensities or long exposures.

Results

When the eye of a grasshopper is illuminated with a short flash of light there ensues a period of time during which nothing happens, followed by a gradually developing negativity which reaches a crest and then declines (Fig. 1). If the flash duration is maintained constant and the intensity is decreased, a series of responses are obtained of decreasing magnitude and increasing latency. Similar series of responses have been obtained with a variety of flash durations.

1. The effect of intensity and duration of illumination on the retinal action potential magnitude.

The data obtained from one grasshopper maintained in the dark at 30°C are presented in Fig. 2. The dots of Fig. 2 relate peak magnitude of the retinal action potential to the logarithm of the flash duration, measured in seconds, at five different intensities. The response magnitudes tend to reach a constant value at flash durations of 0.01 seconds or longer for all values of intensity. Similar data were obtained in 15 other experiments.

The data obtained from one isolated ocellus of Limulus are presented in Fig. 3. The dots of Fig. 3 relate peak magnitude of the retinal action potential (Fig. 1, lowest record) to the logarithm of the flash duration. The short life of the excised preparation limited the data obtainable. Similar results have been obtained in 11 other experiments with isolated ocelli of Limulus.

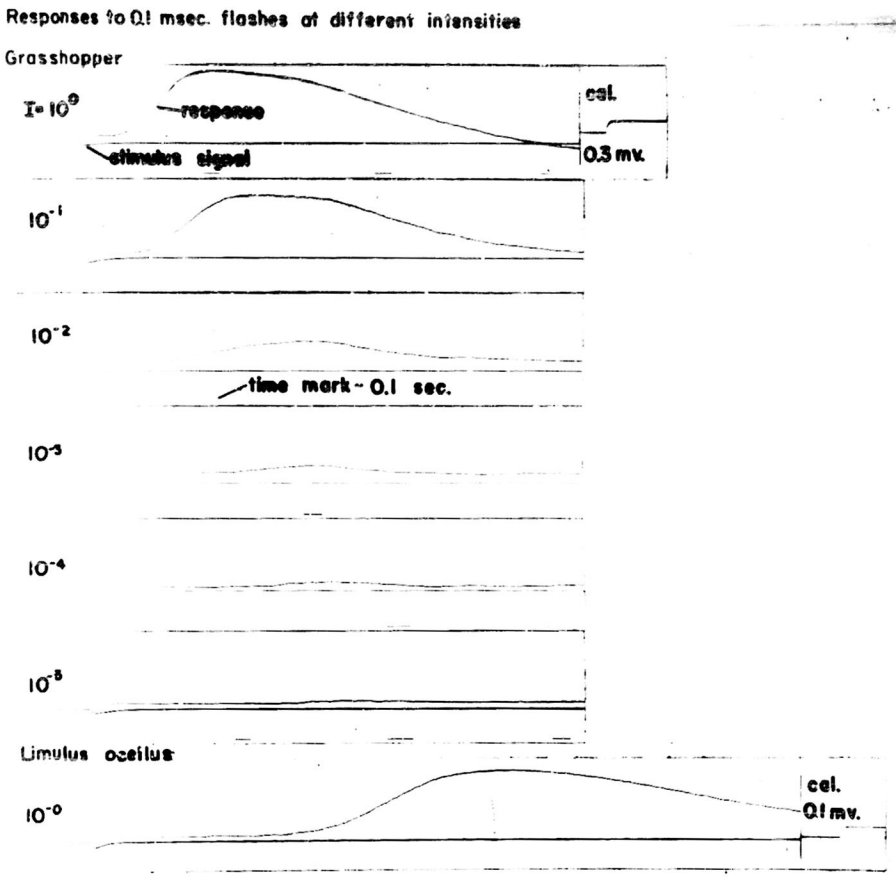

Fig. 1 A series of electrical responses recorded from the eye of a grasshopper (Malanoplus differentialis) illuminated with flashes of 0.1 milliseconds duration and varying intensity. The lowest record indicates the kind of response recorded from the median ocellus isolated from Limulus. The recording paper speed for the grasshopper records was 50 cm. per second; for the Limulus ocellus, 25 cm. per second.

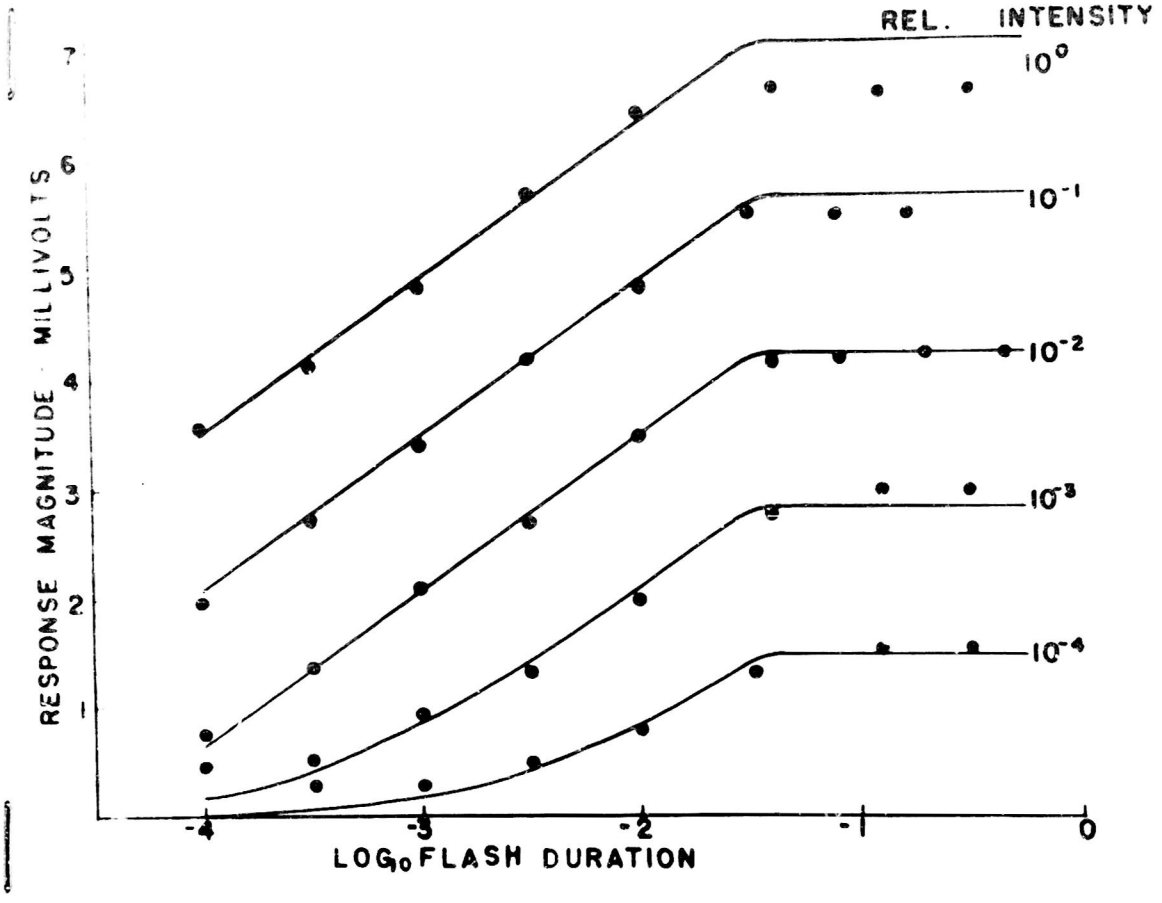

Fig. 2 The closed circles represent the maximum voltage recorded from grasshopper eyes in response to illumination with different intensities and exposures. The curves represent the theoretical relationship between voltage, intensity and flash duration given by expression (5) of the analysis.

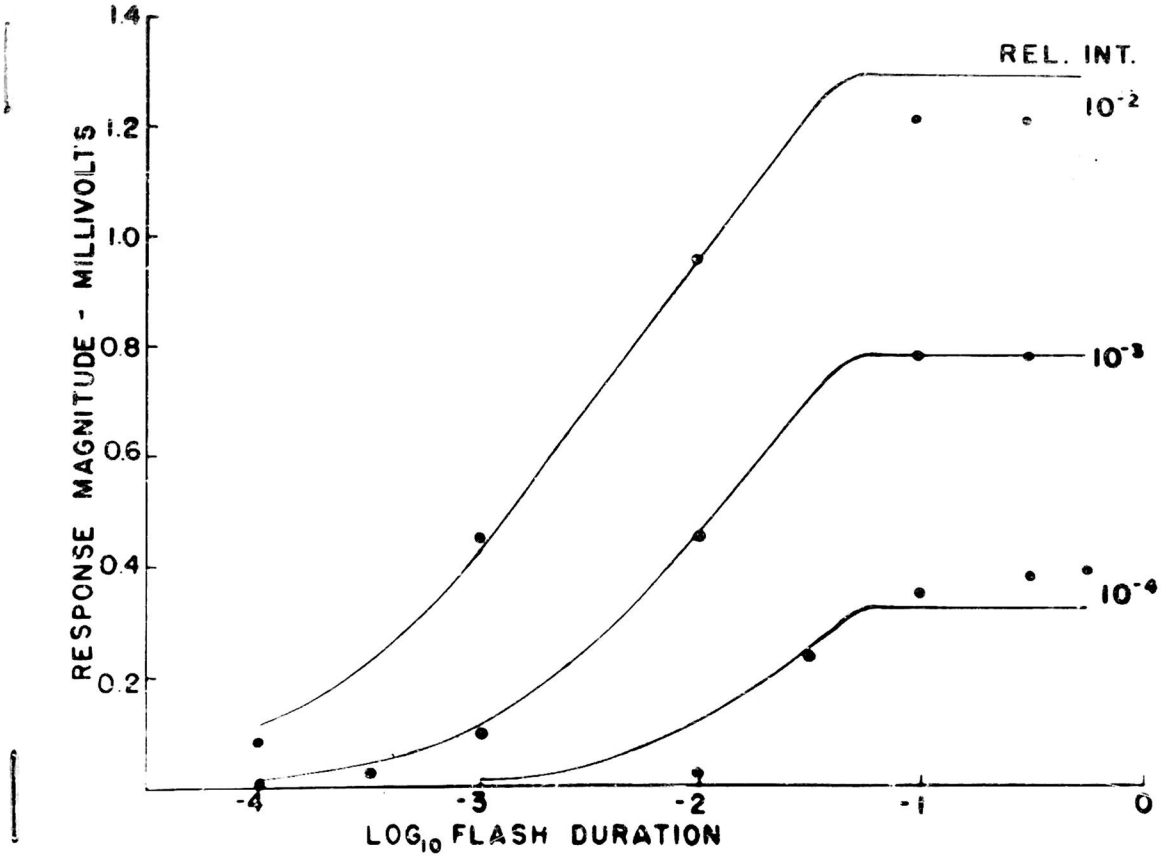

Fig. 3 The closed circles represent the maximum voltage developed by the isolated ocellus of Limulus in response to illumination of varying intensity and duration. As in Fig. 2, the curves represent the theoretical relationship between voltage, intensity and duration of illumination.

2. The effect of intensity and duration of illumination on the latency of the retinal action potential.

The data obtained from one grasshopper maintained in the dark at 20°C are presented in Fig. 4. The data, represented by the circles and crosses, are the latent periods in seconds plotted as a function of the logarithm of the intensity for different flash durations. The latency exhibits a progressive increase as the intensity is decreased (see also Fig. 1.). At any one intensity, the latency increases as the flash duration decreases. The change in latency when the flash duration is decreased from 0.1 sec. (flash duration greater than the latent period) to 0.01 sec. is very slight. For changes in flash duration from 0.01 sec. to 0.001 sec. and from 0.001 sec. to 0.0001 sec. the changes in latency are considerably greater. However, the percentage change in latency from exposures of 0.1 sec. to 0.0001 sec. is nevertheless very small compared to the thousandfold change in the exposure.

Latent period measurements made on the records obtained from the isolated median ocellus of Limulus exhibit the same relationships as the grasshopper data.

Discussion

1. Magnitude

The experimental results on response magnitude as a function of flash duration and light intensity represented for example in Fig. 2 (grasshopper) present a consistent and reproducible picture from one preparation to another. It is apparent that a mathematical description can be checked quantitatively. The following analysis yields a result which is in quantitative agreement with the experimental observations.

The features of the data which a satisfactory theory should correlate are: (1) a lateral displacement of any curve will bring it into coincidence with any other (below the plateau region) and coincident points correspond to equal amounts of electromagnetic energy; (2) above some value of response ($\simeq$ 2 millivolts) and below the plateau region the curves become straight lines; and (3) a bending over to a constant response region occurs (at a relatively fixed value of the flash duration except for the highest intensities).

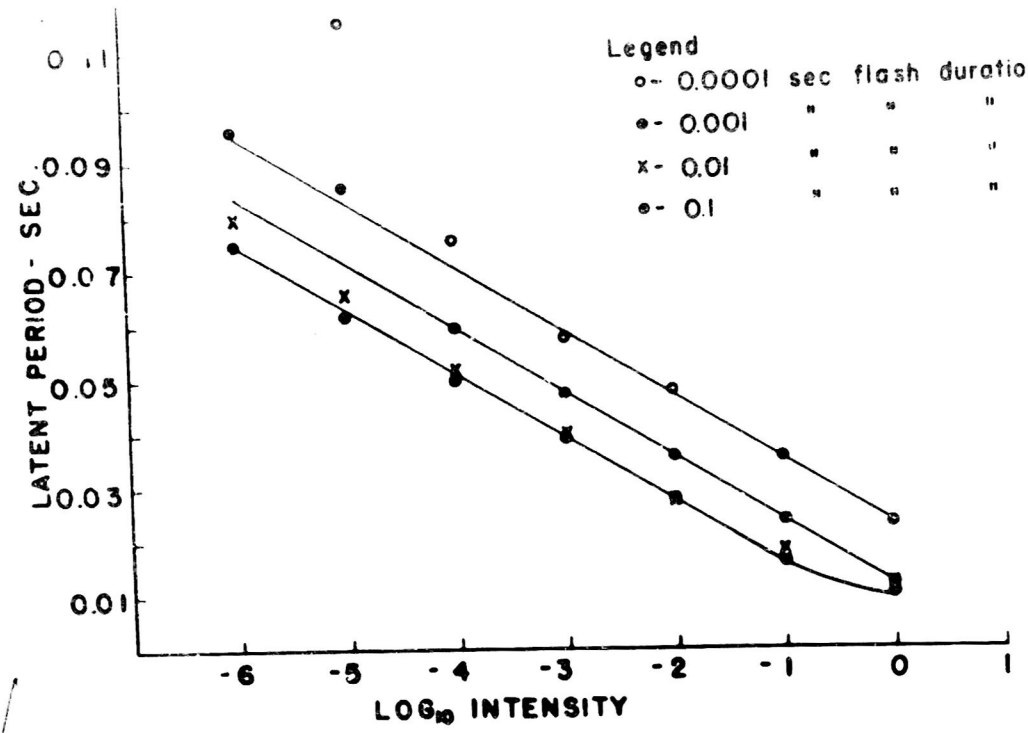

Fig. 4 The circles and crosses represent the latent period of the retinal action potential of the grasshopper plotted as a function of the logarithm of the intensity with the duration of exposure as a parameter. The curves are the theoretical relationship between latent period, intensity and flash duration given by expressions (17) and (27) of the analysis. The theoretical curve corresponding to the experimental points for 0.01 seconds flash duration (crosses) has been omitted because it is so near the curve for 0.1 second flash duration as to be almost indistinguishable on a graph the size of the figure.

The following is an analysis which correlates data of the type illustrated in Fig. 2. The capital letters designate substances or factors and lower case letters designate concentrations or magnitudes. We assume that the exposure of the substance S to light produces a substance C. (Wald, 1951). Let the time rate of converstion of S be given as follows:

(1) $\frac{ds}{dt} = a\,I$

This implies that the amount of S present is not appreciably depleted during illumination. Let N molecules of C follow from one of S. Assume that the concentration of C changes in accordance with the following expression,

(2) $\frac{dc}{dt} = b\,I - K\,(c - c_1)$

where b is proportional to Na. Let the concentration of C in the dark adapted eye be c_1. If the light is flashed on at t = o and off at $t = t_f$ then from (2) it follows that, in the region in which S is transformed, the concentration of C at a time equal to or greater than t_f, is

(3) $c = c_1 + \frac{b\,I}{K}\left(1 - e^{-K t_f}\right)$.

If k = 0, that is if there is no depletion of Substance C, equation (3) is repleased by

(4) $c = c_1 + b\,I\,t_f$.

We assume that the magnitude of the voltage difference measured across the retina is proportional to the logarithm of the ratio of c to c_1. This is expressed as follows:

(5) $E = \alpha \log\left[1 + \frac{b\,I}{c_1 K}\left(1 - e^{-K t_f}\right)\right]$

where E is the voltage difference and α is a proportionality constant. Formula (5) expresses a relationship between voltage, flash duration and light intensity of the type shown in the plot of Fig. 2

If $K t_f << 1$ expression (5) reduces to

(6) $E = \alpha \log\left[1 + \frac{b\,I\,t_f}{c_1}\right]$

Under the condition $Kt_f << 1$ we see from (6) that all curves of E versus log t_f with I as a parameter are identical in shape since (6) shows that E is constant if t_f is multiplied by any factor and I is divided by the same factor. A constant amount of energy is required to produce a constant response magnitude, E.

If, in addition $\frac{bIt_f}{c_1} >> 1$ we obtain

(7) $$E = \alpha \log \frac{bIt_f}{c_1}$$

that is, the relation between E and log t_f is linear in agreement with the experimental results of Fig. 2.

Now as the flash time increases, i.e., $t_f \to \infty$ the concentration of C approaches the equilibrium value given by the following

(8) $$c = c_1 + \frac{bI}{K}.$$

The expression for the response magnitude at equilibrium in terms of voltage thus becomes

(9) $$E = \alpha \log \left(1 + \frac{bI}{Kc_1}\right).$$

We note that if $\frac{bI}{Kc_1} >> 1$ then (9) reduces to

(10) $$E = \alpha \log \frac{bI}{Kc_1}.$$

This expression shows that for intensities such that the above inequality is valid then for equal increments in log I the maxima of the response magnitude increase by equal increments of voltage. However, (9) shows that for low intensities the increments between response maxima will decrease to zero. It also follows from relation (5) that the bend in the curves of voltage versus log t_f will occur at the same value of t_f for all intensities.

The theory developed thus far has not included any appreciable depletion of the substance S; that is, the quantity, a, of expression (1) is a constant. The inclusion of this factor into the theory is readily achieved. It has the effect

of bringing the maxima of the response curves closer together as the intensity becomes large and consequently moving the bend in the curves to the left. This is in accordance with the higher intensity data of Fig. 2. It is, of course necessary to take account of the lowering of the concentration of the substance S when considering the equilibrium response magnitude under conditions of prolonged illumination (Fry and Alpern, '46).

The curves superimposed on the grasshopper eye data of Fig. 2 are theoretical, based on formula (5). It is seen that only at the two highest intensities is there a deviation of the theoretical curves from the experimental values and the discrepency can be removed by taking into account the appreciable depletion of the substance S.

The deviation between theory and data at the lower end of the curved portions of Fig. 2 we believe to be caused by a second potential wave from the grasshopper eye which dominates the electrical response at low intensities and short durations (see Fig. 1). The median ocellus of Limulus, which has a uniform sense cell population, produces a response which exhibits no duality, thus indirectly supporting our belief.

The response magnitude data on Limulus ocellus can be correlated with the same theory as seen from Fig. 3. Theoretical curves are shown superimposed on the somewhat meager experimental data.

In order to compare the theory with experimental results it is necessary to evaluate the three constants, α, K and b/c_1, appearing in relation (5). This can be done conveniently as follows. A set of experimental curves is drawn through the data graphed as in Fig. 2. The quantity α is equal to the slope of the linear portions of the curves of response magnitude versus log flash duration. Symbolically $\alpha = \Delta E / \Delta \log t_f$. The rate constant K can be evaluated by determining the value of the flash duration, t_{fo}, (from the same curve) corresponding to the transition from a linear rising portion to the plateau region for the intermediate intensity range. In practice the two straight lines are extended until they intersect. The value of the flash duration corresponding to this

intersection point is related to the rate constant by the formula $K = 1/t_{fo}$. The value of b/c_1 can then be determined from the plateau value of the response magnitude for any one intensity (excepting the highest intensities) by using relation (9).

2. Latency

Next let us consider the latent period preceding the electrical response of the dark adapted sense cells to a single flash of light. A typical set of experimental data obtained from the eye of a grasshopper is designated by the symbols in Fig. 4.

We will divide the latency theory into two parts. The first analysis is sufficient to yeild the experimentally observed linear portions of the relation between latent period and logarithm of the intensity. In order to explain the asymptotic approach to a finite latent period as the intensity becomes large it is sufficient to introduce a factor into the analysis which accounts for the depletion of the substance S. This is done in the second part of the analysis.

Consider that when substance S undergoes a transformation with light according to relation (1) that a substance or factor, P, changes in accordance with the following relation:

(11) $\frac{dp}{dt} = kp + nI$

where n is proportional to the concentration of S and p is the concentration or magnitude of the factor P. It can be seen from (11) that it is assumed that the generation of P is autocatalytic. If we assume that at $t = 0$ $p = 0$ then one obtains the following solution to (11)

(12) $p = \frac{nI}{k}\left[e^{kt} - 1\right]$.

At the end of the flash, $t = t_f$, and the magnitude of p at this time is

(13) $p_f = \frac{nI}{k}\left[e^{kt_f} - 1\right]$.

If the latent period is greater than the flash time the equation (11) is replaced by

(14) $\frac{dp}{dt} = kp$.

When $t = t_f$, p is given by (13) and if we assume that at the latent time p has reached some critical value p_c one obtains.

$$(15)\quad e^{ht_L} = \frac{hp_c}{nI}\,\frac{1}{1-e^{-ht_f}}$$

for the relation between latent period, flash duration and intensity. If the flash time is equal to or greater than the latent period then relation (15) is replaced by

$$(16)\quad e^{ht_L} = \frac{hp_c}{nI} + 1 .$$

Relations (15) and (16) are conveniently expressed as follows

$$(17)\quad ht_L = (\ln 10)\log\frac{hp_c}{n\left[1-e^{-ht_f}\right]} - (\ln 10)\log I$$

For a constant flash time expression (17) shows that a linear relation exists

$$(18)\quad ht_L = (\ln 10)\log\left[\frac{hp_c}{nI} + 1\right]$$

between t_L and log I for $t_f < t_L$. When the quantity e^{-ht_f} is small compared to one the latent period is independent of the flash time.

It is seen from formula (17) that the latent period varies only slightly for rather large changes in the flash time.

A comparison of the theory with experimental data indicates that the analysis is in agreement with the data over the linear portion of the experimental relation but the data deviates from the linear relation sooner than the theory indicates. The discrepancy is removed in the analysis which follows. It is shown that the asymptotic approach of the latent time to a value not zero at high intensities can be associated with the appreciable depletion of the light sensitive substance S.

The two constants h and p_c/n appearing in formulas (17) and (18) are determined from the single linear relation obtained from the experimental data for flash durations equal to or greater than the latent period. The slope m of the line yields h

$$(19)\quad m = -(\ln 10)/h .$$

The intercept I_i on the horizontal axis yields p_c/n

$$(20)\quad I_i = hp_c/n .$$

We will now modify the analysis for the latent period to take account of the appreciable depletion of the light sensitive substance S which occurs at high intensities and long flash durations.

The expression (1) for the time rate of change of the concentration of S is replaced by

(21) $\frac{ds}{dt} = -\beta I s.$

At t= 0 s= s_0 and the amount of S converted at any time is

(22) $S_0 (1 - e^{-\beta I t})$.

Expression (11) is then replaced by

(23) $\frac{dp}{dt} = hp + \delta \frac{d}{dt} [S_0 (1 - e^{-\beta I t})]$

which becomes

(24) $\frac{dp}{dt} = hp + \delta S_0 \beta I e^{-\beta I t}$.

Let

(25) $\delta S_0 \beta = n,$

so (24) can be written

(25) $\frac{dp}{dt} = hp + n I e^{-\beta I t}$.

If we assume as before that at t = 0 p = 0 then the solution of (25) is

(26) $p = \frac{n I}{h + \beta I} [e^{ht} - e^{-\beta I t}]$.

If $t_f = t_L$ and $p = p_c$ at $t = t_L$ we obtain

(27) $p_c = \frac{n I}{h + \beta I} [e^{h t_L} - e^{-\beta I t_L}]$.

For low intensities (27) reduces to (16) so that the linear portion of the relation between t_L and log I is unchanged. However, at high intensities expression (27) reduces to

(28) $e^{h t_L} = p_c / \delta S_0$.

which shows that the latency for $t_f = t_L$ approaches the asymptotic value

(29) $\frac{1}{h} \ln (p_c / \delta S_0)$.

An analysis for the case $t_f < t_L$ can be carried out with similar results. However, for comparison with the experimental data on hand (29) suffices. Comparison of the experimental data of Fig. 4 with this analysis is given in Fig. 4. One calculates the constant $p_o/\delta s_o$ from the indicated asymptotic value of t_L at high intensities and then computes t_L from (27) for intermediate intensities. It is thus seen that the depletion of the substance S is sufficient to account for the observed deviation from a linear relation for large intensities.

It is noted from Fig. 4 that the experimental data for the shortest flash durations and lowest intensities deviate from the linear relation indicated by the theory. This is probably associated with the difficulty of measuring the latency from the tape records at low response magnitudes. As the magnitude of the response becomes small the error introduced in determining the position on the record at which a deviation from the base line occurs becomes larger and tends to yield greater values of the time. This explanation of the discrepency between theory and data was substantiated by observing that when the gain of the amplifier system was made smaller (resulting in smaller deflections on the tape) the deviation from linearity occured at higher intensities and longer flash durations.

3. Implications

The agreement between theory and fact in the case of the grasshopper retinal action potential may be considered as a strong indication for the existence of two simultaneous coupling processes intermediate between the photochemical and the electrical event in the retina. The analysis has been applied to the data on the electrical response of the isolated median ocellus of Limulus and again general agreement was found in the magnitude and latency (unpublished) relations. Preliminary observations suggest that the response of the lateral eye of Limulus behaves in a manner similar to the grasshopper eye, and the characteristics of other photoreceptors will be examined.

Recently, MacNichols, Wagner and Hartline ('53) presented proof that the

action protential produced by sense cells of the Limulus lateral eye generates the nerve impulse trains in its axon. If the retinal action potential, in general, plays the strategic role in peripheral vision indicated by the work of Hartline and co-workers, then the "coupling" processes producing the retinal action potential assume a sufficient degree of importance to deserve further and intensive investigation.

Wald and co-workers have studied intensively the photochemistry of extracted vertebrate rhodopsin. Wald ('51) describes a photochemical cycle in which there occur two transient substances, lumi-rhodopsin and meta-rhodopsin, which have a very short life at temperatures compatible with life. These substances could play a significant role in peripheral vision. More recently (Wald and Brown, '52) have demonstrated that rhodopsin, illuminated when in an amperometric titration cell, binds silver ions. Various lines of evidence led Wald to suggest that the observed change in concentration was caused by sulfhydryl groups, suddenly uncoupled by the action of light, absorbing cations in the solution being titrated. The time characteristics of the current change were not indicated. An event of this kind, producing an electrical change, could satisfy the theory pertaining to the potential magnitude. What is not apparent at this time is how the latency of the response arises.

It is probable that the elucidation of the potential generating mechanism in photosensitive cells may increase our insight into generator potential phenomena within the nervous system and the living body.

4. Summary

1. The magnitude of the retinal action potential obtained from dark adapted eye of grasshoppers is a function of the intensity and duration of illumination. The form of this relation is consistent from one animal to another.

(a) The characteristics of the relation between action potential and intensity and duration of illumination are reasonably accurately described by a theory which assumes that:

(1) The light acts on a photosensitive substance S producing a material C whose concentration manifests itself as an emf across the retina after the lapse of a latent period.

(2) The time rate of production of C is proportional to the light intensity and the rate of depletion is proportional to the difference between the concentration of C at any time and the equilibrium concentration in the dark adapted eye.

(3) The maximum values of the emf generated are proportional to the logarithm of the concentration of C after illumination.

(b) The experimentally determined relation between response magnitude and logarithm of flash duration, for any single intensity exhibits the following: (i) a slowly rising phase at short flash durations (ii) a linear region at intermediate flash duration and (iii) a plateau region as the flash duration increases further.

(1) In the linear region and below, curves for different intensities show that a constant value of response magnitude is obtained for a fixed value of the product of intensity and flash duration. This characteristic is derivable from the hypothesis that C is generated at a rate proportional to the intensity of illumination and that the emf across the retina is proportional to the logarithm of the concentration.

(2) The magnitudes of the response in the plateau region (i.e., long flash durations) are, for equal logarithmic increments of intensity, uniformly spaced at intermediate response magnitudes, compressed together at low response magnitudes and also compressed together at

high response magnitudes. The existence of the plateau regions is correlated with the depletion process for the substance C. The data are quantitatively described by the theory except for the compression at high response magnitudes. To explain this compression it is sufficient to include in the theory the dependence of the rate of the initial photochemical process on the concentration of the light sensitive substance S, which for high intensities and long flash durations, is appreciably depleted during the flash.

2. The latency of the retinal action potential is, for a single flash duration, a linear function of the logarithm of the intensity over almost the entire range of intensities used in the experiments. The latency is a relatively insensitive function of the flash duration.

(a) The characteristics of the relation between latency and intensity and flash duration are accurately described by a theory which assumes that:

(1) The time rate of production of a factor or state P whose magnitude determines when the electrical response begins is proportional to the intensity of illumination and is also autocatalytic.

(2) When the magnitude of the factor P reaches some critical value the electrical response begins.

(b) (1) The linear portion of the experimentally determined relation between latent period and the logarithm of intensity for a constant flash duration is accurately described by the theory based on an autocatalytic rate process initiated by the light.

The light sensitive substance S is not appreciably depleted.

(2) The latent period varies only very slightly with flash duration as the flash duration becomes less than the latent period. As the flash duration further decreases the latent period changes more rapidly but still quite slowly compared to the changes in flash time. This characteristic of the latency is quantitatively described by the theory just mentioned.

(3) The experimental data show, for the highest intensities and longest flash durations, that the latent period is no longer a linear function of the logarithm of the intensity. As the intensity increases the latency appears to approach a finite non-zero value. This aspect of the latent period can be explained by modifying the theory to take account of the appreciable depletion of the light sensitive substance S, which occurs at high intensities and long flash duration.

3. The characteristics of the retinal action potential obtained from the isolated median ocellus of Limulus are similar to the characteristics of the grasshopper responses and are in agreement with the theory.

III. (b) Retinal action potential - theory and experimental results for the eye of frog and Limulus.

The agreement between the two factor theory for retinal processes and the data obtained from the latent complex eyes of grasshoppers was found to be satisfactory (see III a). To test the theory more vigorously it was decided to examine the electrical behavior of the lateral eye of Limulus and the eye of the frog. The experimental results are presented below and their correspondence with the theory is indicated.

Methods

1. The conduct of the experiments with Limulus was essentially similar to the grasshopper experiments (III b). The entire animal was mounted in a dark chamber containing sea water through which oxygen was circulated. The temperature was maintained at 20°C $\pm$ 0.2°C.

2. The conduct of the experiments on the frog eye deviated from the previous experiments as follows:

(a) the frogs were injected with d-tubocurarine, the dosage varying from 0.03 to 0.045 mg. per Kg wet body weight. This procedure was found necessary to immobilize the animals.

(b) the entire animal was mounted in a chamber containing water, through which oxygen saturated with water vapor was circulated.

(c) the temperature was maintained at 15°C $\pm$ 0.2°C. This low temperature was selected to decrease the metabolic rate of the curarized animals and to prolong the effects of the drug.

(d) the active electrode was a small pipette with a tip of about 0.2mm and filled with agar made up in amphibian Ringer's solution. The electrode was inserted into the vitreous through a hole in the sclera just behind the iris toward the anterior side of the pupil. The inactive electrode consisted of a wick laid on an exposed dorsal portion of the eyeball.

Results

(a) The relation of magnitude of the retinal action potential to the intensity and duration of the light stimulus.

1. The lateral eye of Limulus.

The results of one experiment with the lateral eye of Limulus are presented in Fig. 5. The magnitudes of the retinal action potential (dots) are plotted as a function of the common logarithm of the duration of the light stimulus, for different values of intensity. The data constitute a family of curves. At the intensity $I = 10^{-6}$, only one response was recorded. The continuous lines on the graph represent the theoretical prediction. The dashed line connects one set of data which are not yet adequately predicted by the theory. Five experiments have been performed on the lateral eye of Limulus, all yielding essentially the same result.

2. The eye of the frog.

The curarized frog preparation at 15°C and in an atmosphere of oxygen will remain immobilized about 24 hours. Supplementary injection of curare was attempted but was discontinued because of variations in response characteristics subsequent to injection. Consequently, two sets of data from frogs are presented. In Fig. 6 the response magnitudes of the frog eye to flashes of low intensity are presented, plotted as a function of the common logarithm of the flash duration. The dots represent experimental data and the continuous lines the theoretical prediction. The dashed lines have been drawn through points which are not predicted by theory. In Fig. 7 the response magnitudes of the frog eye to flashes of higher intensity are presented, the data plotted as in Fig. 6. The dots represent the experimental findings, continuous lines are theoretical and the dashed lines connect data not adequately covered by the theory. Eleven experiments have been performed on curarized frog eyes; all yielding essentially the same results.

(b) The relation of the latent period of the retinal action potential to the intensity and duration of stimulation.

1. The lateral eye of Limulus

The latent periods obtained in one experiment are presented in Fig. 8, plotted as a function of the common logarithm of the light intensity, for different values of flash duration. The dots represent experimental findings, the lines represent the theoretical prediction. Latent periods obtained in other experiments show similar relationships to intensity and flash duration.

2. The eye of the frog.

The latent periods obtained in two frog experiments are presented in Fig. 9, plotted as a function of the common logarithm of the light intensity, for different flash durations. The dots represent the experimental findings and the lines represent the theoretical expectations. Results from all other frog experiments are similar.

Discussion

1. The retinal action potential magnitude.

The following formulae were employed to compute the theoretical predictions:

1) $E = \alpha \log \left[1 + \frac{bIt_f}{c_1}\right]$, for the curved rising phase of the magnitude vs. $\log_{10}$ flash duration curves. In this equation E represents the magnitude (in millivolts) of the retinal action potential; I is the relative intensity; t_f is the flash duration; α is a constant computed from the slopes of the linear portion of the data and $\frac{b}{c_1}$ is a constant computed from the intercepts on the x-axis of the linear portion of the data.

2) $E = \alpha \log \frac{bIt_f}{c_1}$, for the linear rising phase of the magnitude vs. $\log_{10}$ flash duration curves.

3) $E = \alpha \log \left[1 + \frac{bI}{c_1 K}\right]$, for the plateau region of the response magnitude -$\log_{10}$ flash duration curves. The constant K is equal to the reciprocal

of the flash duration where the rising phase of the response curve intersects the pleateau curve for the same intensity.

The continuous lines in figs. 5, 6 and 7 represent the predictions based on equations 1, 2 and 3. The dashed lines represent data which is not predicted by the current form of the theory. The deviation from theory is two-fold; first, the slope of the rising phase of the response curves for high intensities ($I = 10^0$ for the Limulus eye, and $I = 10^{-2}$ for the frog eye) have a slope significantly lower than the slope of the curves for lower intensities; secondly, the increments in the plateau magnitudes for equal increments of I decrease much more rapidly than the theory predicts. The latter deviation was also apparent in the grasshopper data (III a). Extension of the theory relating to potential magnitude to include the depletion of photosensitive material in the photoreceptor during the incidence of a flash of light, led to an approximation which satisfactorily accounted for the reduction in plateau increments. A rigorous extension of the theory is underway and, when complete, will be tested in terms of the high intensity data from frog and Limulus eyes.

Comparison of the response magnitude characteristics of the grasshopper, horseshoe crab and frog eye indicates the following: 1) the characteristics of the two compound eyes, grasshopper and horseshoe crab, are quite similar; 2) the characteristics of the frog eye differ considerably from either the grasshopper or the Limulus eye in the following respects: a) the plateau magnitudes obtained from the frog eye shows compression at intensities that are still relatively low ($I = 10^{-5}$) compared to the grasshopper and horseshoe crab, and b) the critical duration, i.e. the shortest flash duration which will elicit the maximal response for any given intensity decreases rapidly as the intensity increases (Fig. 6). The long critical durations at low intensity enables the vertebrate photoreceptor to produce responses of greater magnitude than the compound eye under similar circumstances. Conceivably, this may make the frog eye a better light detecting device than the compound eye under conditions of low illumination.

2. The latent period.

The following formulae were used to compute the theoretical predictions.

4) $ht_L = \ln \frac{hPc}{n\left[1 - e^{-ht_f}\right]} - \ln I$, for the linear portion of the data. In this equation, t_L is the latent period, t_f the flash duration, I is the intensity, Pc is the critical concentration of factor P, which permits the response to occur, h and n are constants. The constant h is determined from the slope of the line connecting the data for flash duration of 0.1 sec. The constant $\frac{hPc}{n}$ is determined from the intercept of the above line with the x axis.

5) $Pc = \frac{nI}{h + \epsilon I}\left(e^{ht_L} - e^{\epsilon I t_L}\right)$, for the curved portion of Fig.9. The symbols as in equation 4 and ϵ is an additional constant. ϵ is evaluated from the estimated asymptotic value of the latency -log I curve for flash durations of 0.1 sec. at high intensity.

The agreement between theory and fact is satisfactory. The data obtained from the frog eye deviate sharply from the predicted value at low intensities and short flash durations. This deviation may perhaps be attributable to low response magnitudes and attendant difficulties in making accurate measurements of the latent period.

The question arises whether the critical flash duration might not be related to the shortest latent periods recorded for any given intensity, i.e. whether only the light incident on the receptor during the latent period might affect the response magnitude. Comparison of the critical flash durations and minimal latencies at corresponding intensities shows that there is a high degree of correspondence in the Limulus data, but no apparent correspondence in the data from the eye of the grasshopper or the eye of the frog. We conclude, therefore, that the critical duration is independent of the latent period and dependent upon the intensity and the rate of the depletion reaction for the electro-motively active material.

III c The effect of temperature upon retinal processes.

According to the theory pertaining to the retinal action potential magnitude, the critical flash duration and the plateau magnitude is dependent upon the intensity and rate of the reaction causing the depletion of the electromotively active material.

According to the formula

6) $\frac{dc}{dt} = bI - K(c - c_1)$, since $E = \alpha \log \frac{c}{c_1}$,

if we maintain I constant and vary the rate of the reaction $K(c-c_1)$, we should be able to affect the plateau magnitude; if the rate of the reaction is decreased, the plateau magnitude for constant I should increase; if the reaction rate is increased, the plateau magnitude should decrease for the same value of I. One of the easiest and perhaps most physiological ways of manipulating reaction rates is by varying the temperature of cold blooded organisms. Such temperature experiments form the subject of this section.

Method

The temperature experiments were performed on intact grasshoppers, using the technique described in III a. Temperature was maintained at 10°C, 20°C and 30°C for periods long enough to obtain a complete series of results. At any level the temperature was constant within $\pm$ 0.2°C.

Results

1. The retinal action potential magnitude.

The results of the temperature studies are exemplified by the data of Fig. 10. Here response magnitude is plotted as a function of the common logarithm of the flash duration, for different temperatures. The intensity was held constant at $I = 10^{-2}$. The magnitude data in the rising phase were sufficiently similar that a single curve was drawn to describe the results at all three temperatures. The plateau values were significantly different, as shown. The best line was drawn through the plateau values for each temperature and was purposely extended to intersect with the rising phase of the curve.

The constant K of equation 6 (and also of equation 3) is equal to the reciprocal of the flash duration at the intersection of the rising limb and plateau limb of the response curve. The common logarithm of k was determined and this value was plotted as a function of the reciprocal of the absolute temperature (see Table 1). From the slope of the resulting curve the activation energy for the depletion reaction was found to be 12,010 cal.

Table 1

The common logarithm of the constant K for 3 different temperatures and two intensities of stimulation. Shown also is the computed heat of activation.

Temperature °C	$\frac{1}{T_{abs.}}$	$I = 10^{-2}$ log K	$I = 10^{0}$ log K	ΔE
10°	3.54×10^{-3}	0.94	1.30	1.162×10^{4} $I=10^{-2}$
20°	3.41×10^{-3}	1.30	1.65	1.24×10^{4} $I=10^{-0}$
30°	3.3×10^{-3}	1.60	1.90	Average 1.201×10^{4} cal.

Six other experiments on grasshoppers at different temperatures have given essentially the same results. A set of data similar to the above was obtained using an intensity, $I = 10^{0}$, and log K was determined. (Table 1). The activation energy obtained from this plot was found to be 12,400 cal.

The average energy of activation was found to be 12,010 calories.

2. The latent period.

The dependence of latent period upon the temperature is indicated in Table 2 and 3. Values of latent period are presented for two intensities of stimulation. Three experiments were performed at each intensity, one each at 10°, 20° and 30°C. At each temperature the latent periods are given for responses to four flash duration.

The average temperature coefficient was computed over the range 10-20°C and over the range 20-30°C. for each intensity. (See tables 2 and 3). The overall average Q_{10} for the latent period is 3.24.

Table 2

Values of latent period in seconds, as functions of flash duration and temperature, at relative intensity = 10^{-2}

Temperature	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Average Q_{10}
10°	0.147	0.120	0.104	0.086	3.96
20°	0.037	0.035	0.023	0.022	2.27
30°	0.018	0.012	0.011	0.011	

Table 3

Values of latent period in seconds, as functions of flash duration and temperature, at relative intensity = 10^{0}

Temperature	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Average Q_{10}
10°	0.084	0.066	0.045	0.040	4.06
20°	0.022	0.014	0.012	0.010	2.42
30°	0.009	0.006	0.005	0.004	

Discussion

1. Magnitude.

The behavior of the retinal action potential in the face of altered temperature is exactly as predicted by the theory. During the rising phase of the response curve, when the magnitude is controlled only by the intensity and flash duration, temperature has no effect on the response magnitude. This may be interpreted to mean that, during the rising phase, the magnitude is dependent only upon the rate of a photochemical reaction, whose temperature coefficient is one. In the plateau region, the response magnitude is determined

by the intensity of illumination and by the rate of decay of the material, ϵ_2. If the decay reaction is thermolabile, then the plateau magnitude should be affected, but in a most extraordinary way. A reduction in temperature should produce an increase in the magnitude of the potential and an increase in the temperature should produce a reduction in potential magnitude. The experimental results verify these predictions.

The activation energy computed from the changes in the velocity constant, K, of 12,010 calories represents a value typical of many biological reactions.

2. Latency.

The magnitude of the latent period at constant stimulating energy varies inversely with the temperature. When the temperature increases the latent period decreased and vice versa. The overall Q_{10} for the latent period, over a temperature range 10° to $30^{\circ}C$, of 3.24 suggests that the reaction controlling the latent period is a thermolabile chemical reaction. The theory concerned with the latent period suggests that the latent period controlling reaction is an autocatalytic reaction, a suggestion in general agreement with the observed temperature effects.

The independence of the potential magnitude (rising phase) and the dependence of the latent period upon temperature is indirect support for the dual nature of the theory developed in III a. Clearly, the disparity in the temperature lability excludes the same reaction controlling both retinal action potential magnitude and its latency.

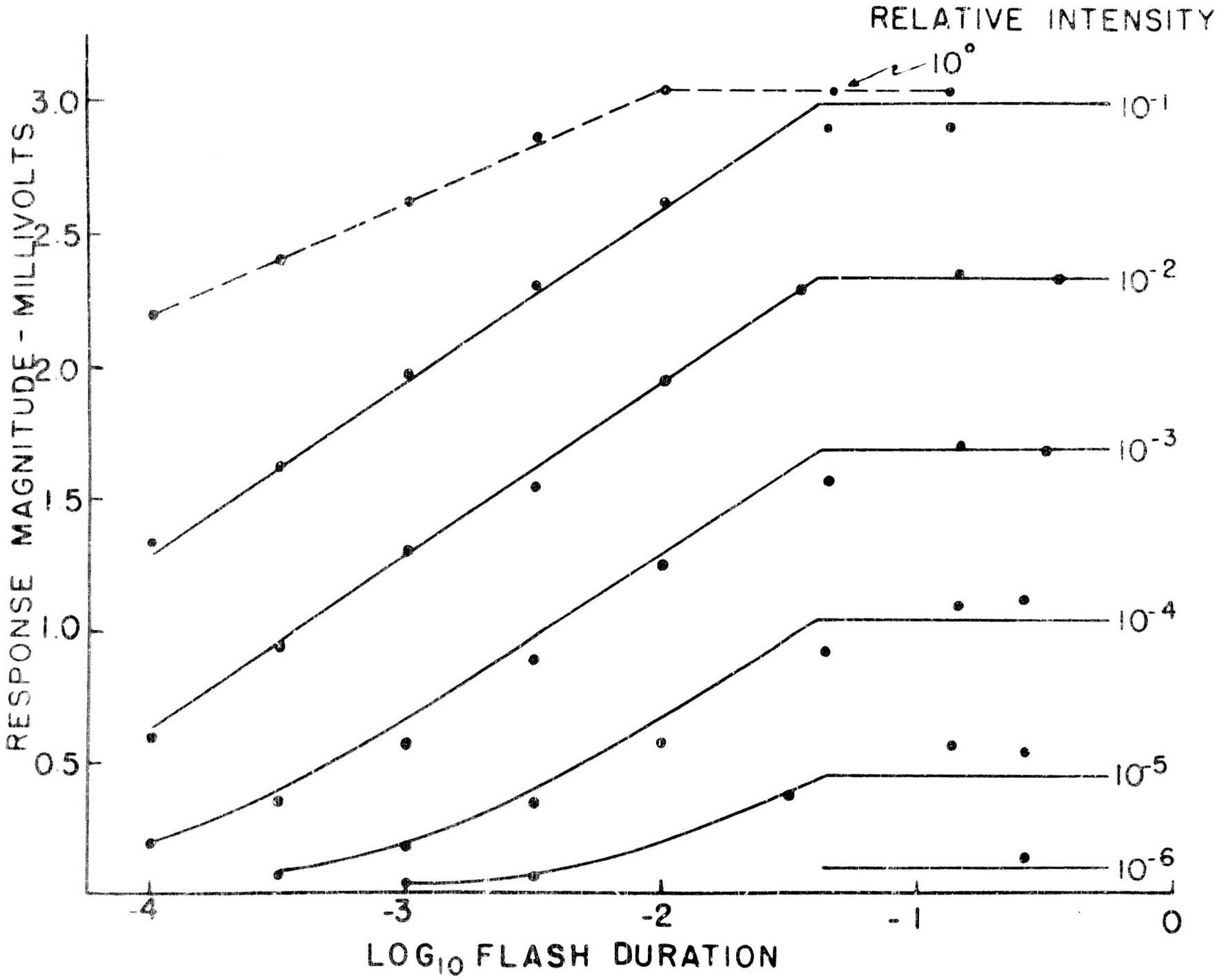
RELATIVE INTENSITY
10°
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
10⁻⁶
RESPONSE MAGNITUDE - MILLIVOLTS
3.0
2.5
2.0
1.5
1.0
0.5
-4
-3
-2
-1
0
LOG10 FLASH DURATION

Fig. 5 The response magnitude (millivolts) of dark-adapted lateral Limulus plotted as a function of the common logarithm of the flash duration. The relative valves of intensity are given to the right of the figure. The dots represent the experimental findings and the continuous lines represent the theoretical prediction. The dashed line connects a set of points which are not adequately predicted by the theory.

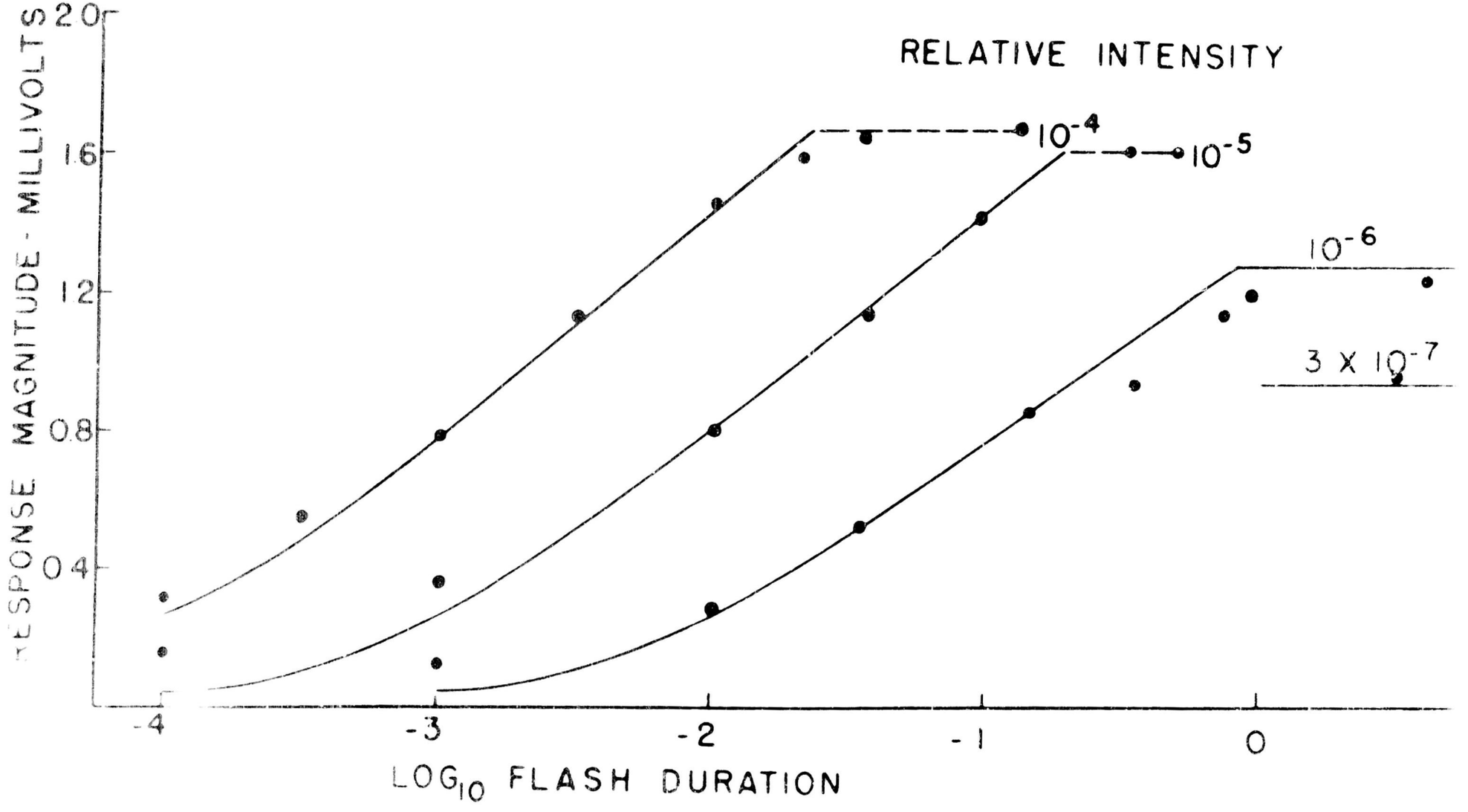
RELATIVE INTENSITY
10^{-4}
10^{-5}
10^{-6}
3×10^{-7}
RESPONSE MAGNITUDE - MILLIVOLTS
20
16
12
0.8
0.4
-4
-3
-2
-1
0
LOG$_{10}$ FLASH DURATION

Fig. 6 The response magnitudes of the dark adapted frog eye plotted as a function of the common logarithm of the flash duration. The relative values of intensity are given to the right of the figure. The dots represent the experimental findings and the continuous lines represent the theoretical prediction. The dashed line connecte points which are not adequately predicted by the theory.

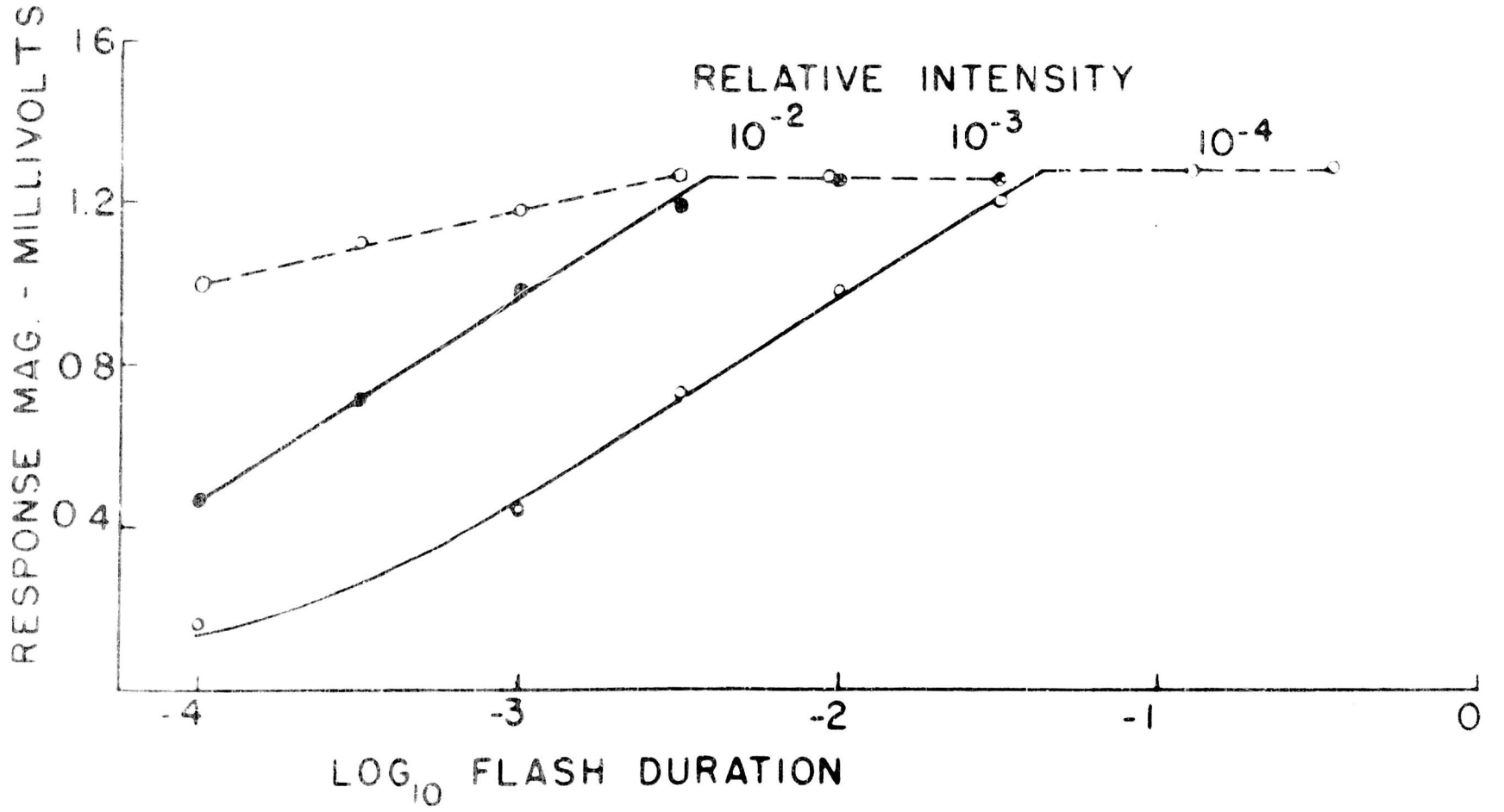
RESPONSE MAG. - MILLIVOLTS
16
1.2
08
04
RELATIVE INTENSITY
10^{-2}
10^{-3}
10^{-4}
-4
-3
-2
-1
0
LOG_{10} FLASH DURATION

Fig. 7 The response magnitudes of the dark adapted frog eye plotted as a function of the common logarithm of the flash duration. The relative values of intensity are given to the right of the figure. The dots represent the experimental findings and the continuous lines represent the theoretical prediction. The dashed line connects points which are not adequately predicted by the theory.

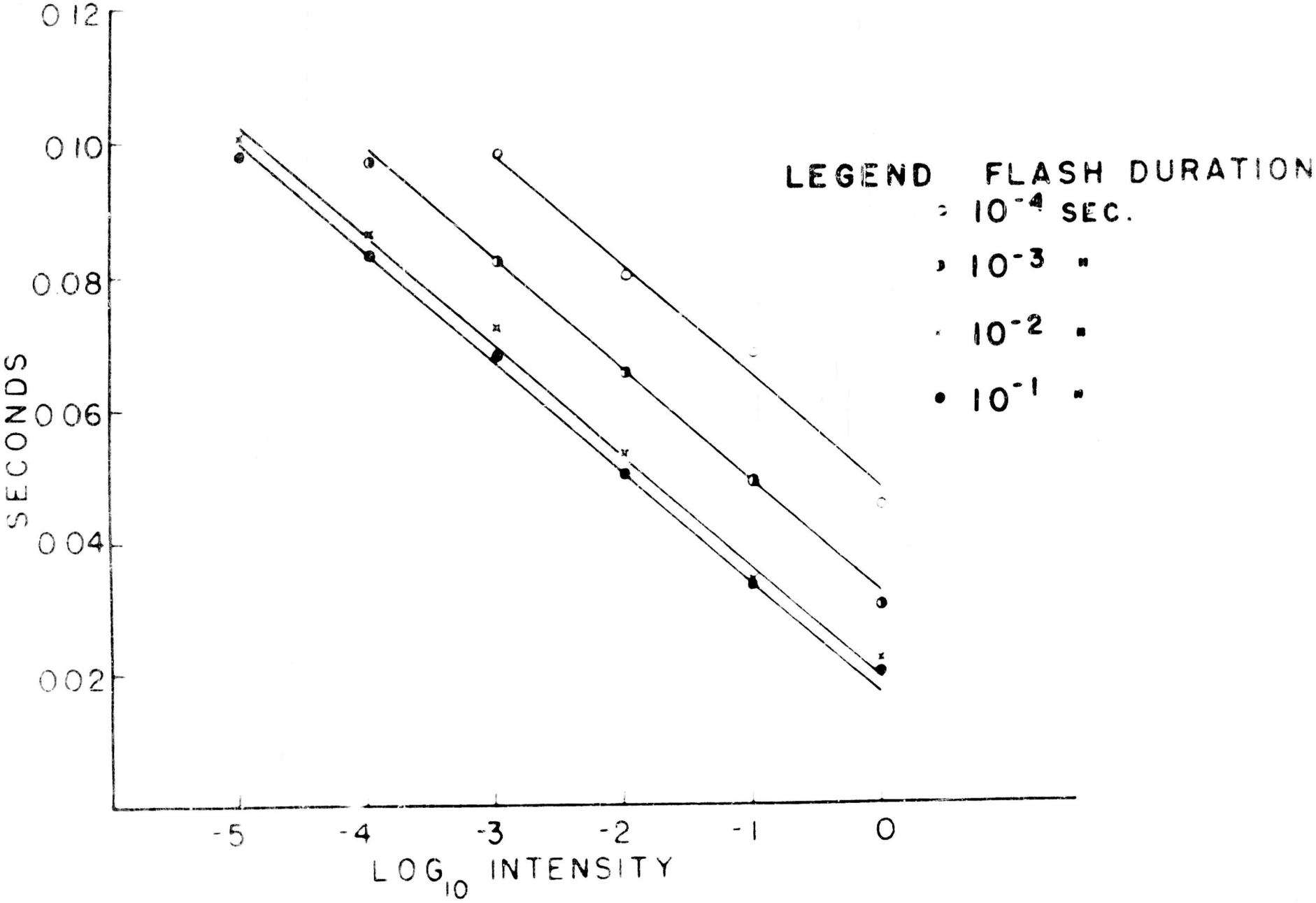
0 12
0 10
0.08
0 06
0 04
0 02
SECONDS
-5
-4
-3
-2
-1
0
LOG10 INTENSITY
LEGEND FLASH DURATION
10-4 SEC.
10-3 "
10-2 "
10-1 "

Fig. 8 The latent periods of the retinal action potential obtained from the lateral eye of Limulus, plotted as a function of the common logarithm of the intensity, for different values of the flash duration. The dots represent experimental observations and the continuous lines represent the prediction of the theory.

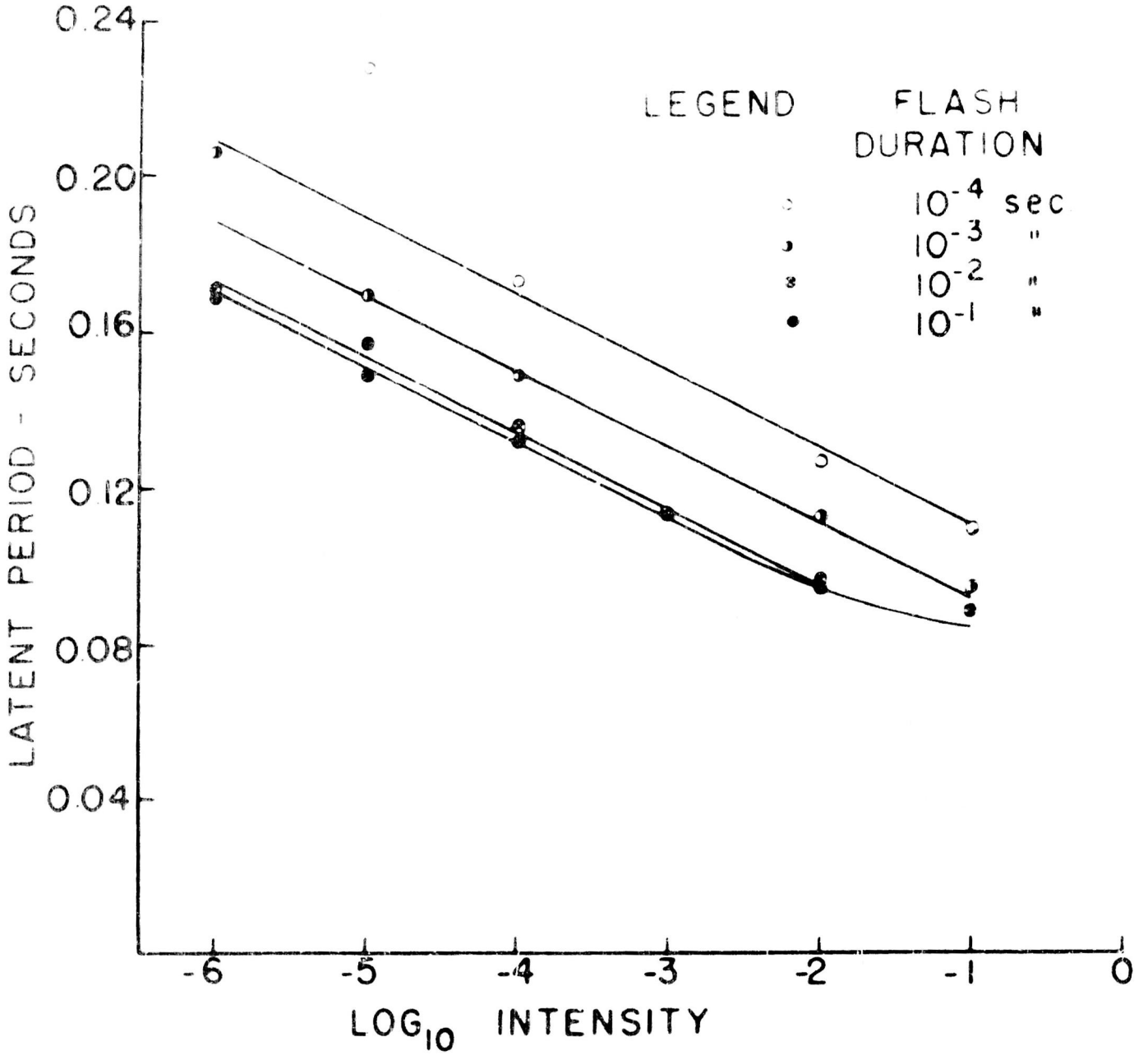
LEGEND
FLASH DURATION
10^{-4} sec
10^{-3} "
10^{-2} "
10^{-1} "
LATENT PERIOD - SECONDS
0.24
0.20
0.16
0.12
0.08
0.04
-6
-5
-4
-3
-2
-1
0
LOG_{10} INTENSITY

Fig. 9 The latent periods of the retinal action potential obtained from the eye of the frog, plotted as a function of the common logarithm of the intensity, for different values of the flash duration. The dots represent experimental observations and the continuous lines represent the prediction of the theory.

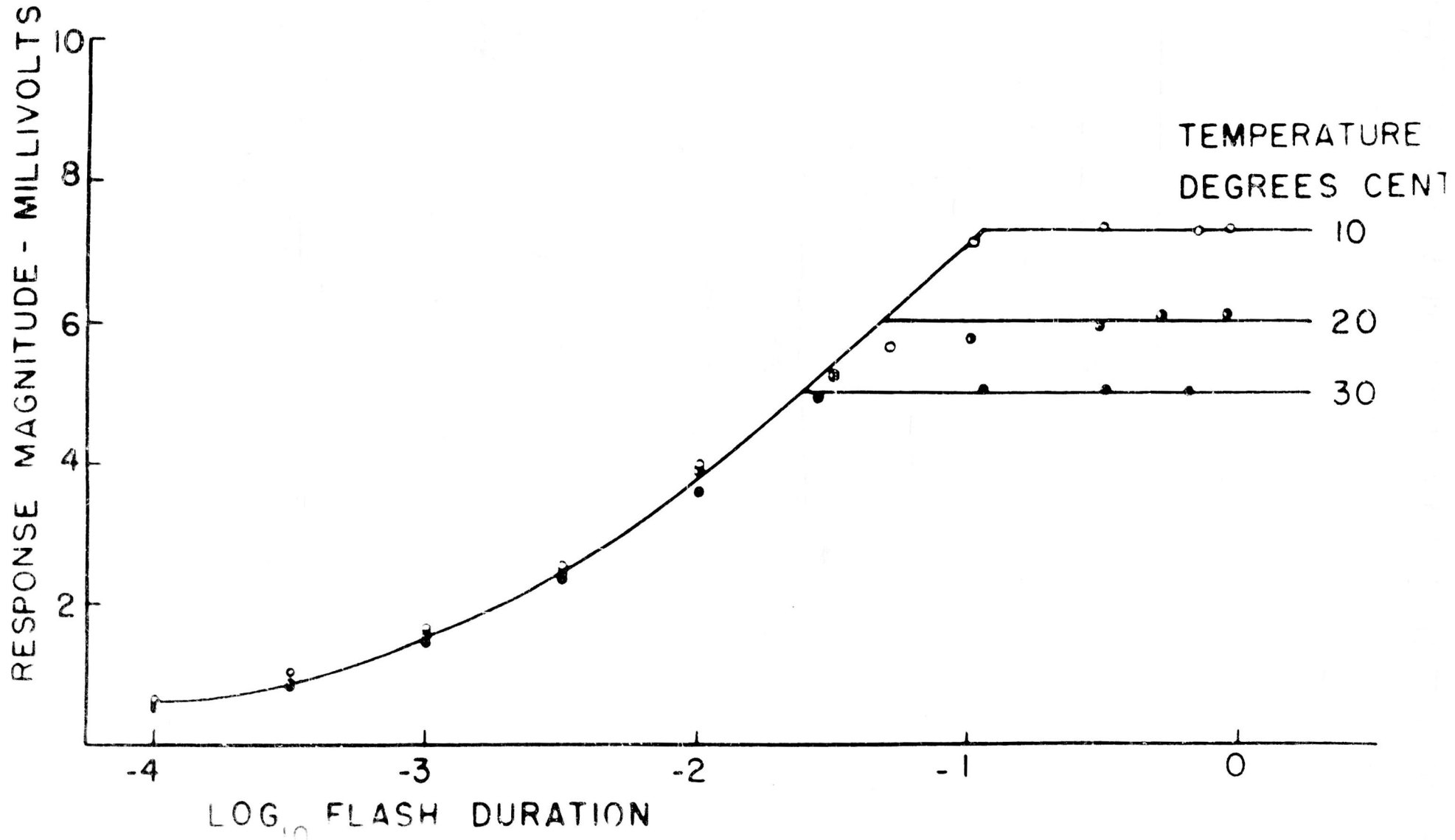
RESPONSE MAGNITUDE - MILLIVOLTS
10
8
6
4
2
-4
-3
-2
-1
0
LOG10 FLASH DURATION
TEMPERATURE
DEGREES CENT
10
20
30

Fig. 10 The response magnitude obtained from the lateral eye of the grasshopper, plotted as a function of the common logarithm of the flash duration, for different temperatures. The curves were arbitrarily drawn to represent the best fit and the plateaus were purposely extended to intersect the rising portion of the curves.

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